INVESTIGATION OF *OCCURRENCE AND LEVEL* OF *ORGANOCHILORINE AND OTHER* PESTICIDES IN THE IAR LAKE, *SAMARU* - ZARIA.

BY

EBELENDU ANTHONY ONYEBUEKE

B.Sc. (Hons) UNN. (1979).

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN ANALYTICAL CHEMISTRY

DEPARTMENT OF CHEMISTRY,
AHMADU BELLO UNIVERSITY
ZARIA.

1982

DECLARATION

I declare that this is my own work and has not been submitted in any form for another degree or diploma at any other University or Institution. Information derived from the published or unpublished work of others has been acknowledged in the text.

Mr. Ebelendu Anthony O.
(M.Sc. Candidate)

Dr. H.T. Bozimo
Ag. Head of Department
(Supervisor)

Dr. G.F.S. Harrison
(Co-Supervisor)

DEDICATION

Dedicated To All Persons and Bodies

Involved In Environmental Protection

ACKNOWLEDGEMENT

I am greatly indebted to my Project Supervisors: Dr. H.T. Bozimo and Dr. G.F.S. Harrison. Dr. Harrison helped me in the design of the water sampling device, and physically assisted in the collection of samples from the lake, a task which would have been impossible for me alone. He also read the draft of this manuscript and made useful suggestions.

The expertise of Dr. Bozimo in gas chromatographic analysis really made this work easier than it would have been without him. He also found time to read the draft of this manuscript meticulously in spite of his congested programme; Dr. Bozimo is the Ag. Head of Chemistry Department and Deputy Dean, Faculty of Science, Ahmadu Bello University, Zaria. I consider it a privilege to have these devoted dons as supervisors.

My thanks also go to Mr. S. Nwokoccha of Plant Pathology Department, IAR, A.B.U. for providing the only pesticide standards available for this work. I also thank Mr. A.F. Smith - the Transport Officer of Chemistry Department, and the departmental drivers for

for their cooperation. I must mention that the good companionship of my colleague; Temi Akporhonor helped in no small way to reduce the boredom of this research.

Finally, I am specially grateful to Dr. C.O. Ikediobi of Biochemistry Department for his assistance at a decisive period of this course, and for his fatherly advice on the choice of project.

A B S T R A C T

This thesis reviews the history of the development and use of pesticides, and of residue analytical techniques. References *are* provided on the distribution, persistence and toxicity of pesticides.

The optimal conditions for gas chromatographic analysis of five organochlorines *and* one carbamate with a Varian Model 3700 Gas Chromatograph were established. The recoveries of five organochlorines in water usinq the EPA method of extraction and clean-up were determined. Disti1led-and-deionized water was analyzed for pesticide residues.

A lake sampling device to be operated from a boat was designed and is described. Description Is also given of sampling techniques designed for lake environments. A pilot sample from the IAR Lake was analyzed for pesticide residues prior to analysis of real lake water samples. Possible sources of error are discussed.

The analytical results *are* compared with documented data on residue levels for both polluted and unpolluted waters, and with permissible levels of residues in potable and surface waters. Suggestions for further work are made;

and, finally, a statement is made on pesticide pollution of the 1AR Lake.

LIST OF TABLES

List of Figures

TABLE OF CONTENTS

TABLE OF CONTENTS(Cont'd)

CHAPTER I

INTRODUCTION

1.1 The Development and Use of Pesticides

Agricultural production decreased greatly during World War II due to shortage of farm labour and farm materials. The acute food shortage immediately after the war made increased food production the priority of many governments. This necessity provided the impetus for research chemists to find substances capable of controlling plant and animal pests so that the required level of food production could be realised. The result was the advent of the wide-scale use of pesticides which yielded astounding results under the focus of military need.

The history of chemical pest control began in the middle of the ninenteenth century during the invasion by Colorado Beetle of potato farms in the United States. The farmers took the unprecedented step of using arsenical poisons for pest control. Since then, the development of insecticides has made steady progress.

About 1850, two important natural insecticides were introduced: rotenone from the roots of the derris plant, and pyrethrum from the lower heads of a species of chrystenthemum. About this time too, soap was used to kill aphids , and sulphur was used as a fungicide.

The discovery of the pesticidal potency of Bordeaux mixture was made accidentally by Millardet in 1882. After daubing roadside vines with a mixture of copper sulphate and lime to discourage pilfering of the crops, he found that these crops were free from the downy mildew dissease which infected the undaubed vines away from the roads. He then conducted experiments which established the effectiveness of the Bordeaux mixture (copper sulphate, lime, and water) against vine mildew.

Subsequent years saw the introduction of materials containing copper, mercury, and sulphur. The development of industrial fungicide, was stimulated by the growth of railways and the consequent need to protect wooden railroad ties from rot. Among the first to be used was creosote which consists of salts of mercury, zinc, and copper.

In fact, many of the well-known poisons have been used for pest control at one time or the other. Cyanide was generated as hydrogen cyanide to kill bed bugs and wood

boring beetles in buildings, while arsenate was used for controlling boll weevil in cotton.

The Advent of Synthetic Organic Pesticides

The 1930s saw the beginning of the large scale use of synthetic organic pesticides. Of the insecticides, the organochlorines stand out as the oldest and the most widely used. The first to be used and, later the most controversial member of this group is DDT.

DDT was first synthesized by the German chemist, Zeidler in 1874 [1], but its properties as an insecticide were not discovered until 1939. Its insecticidal properties were discovered in the Basle laboratories of the Swiss company of J.R. Geigy by Dr. Paul Muller in the autumn of 1939, and patent protection was made in Switzerland on 7th March 1940. Muller was later awarded a Nobel Prize for his discovery. However, it was late in 1942 when World War II was at its height that DDT was brought to the attention of the Allies. Following successful initial field tests in Switzerland against Colorado Beetle, it was manufactured in 1943 and soon became the most widely used single insecticide in the world.

BHC (benzene hexachloride) was first prepared by the English chemist, Michael Faraday in 1825, though its insecticidal properties were not recognized until 1942. It was produced in 1942 in England by workers in I.C.I. laboratories who were searching for a substance to kill Turnip Flea Beetle. [2]

Thiodan was an experimental insecticide developed in Germany by Farbwerke Hoechst. Lindquist and Dahman later separated the technical sample into two isomers A and B.

From 1945 several insecticidal chlorinated hydrocarbon cyclodiene compounds were introduced and they gained widespread use by the middle of the 1950s. These included aldrin, endrin, dieldrin and heptachlor.

The organophosphorus group of insecticides was developed by Schrader in Germany [3]. The development of the compounds stemmed from wartime research on nerve gases for use in chemical warfare when it was discovered that they showed great promise for this purpose.

A closely related group of insecticides, the carbamate esters, was first discovered by the Geigy company in Switzerland in 1947. However, the most generally effective member of the group - carbaryl or sevin was not introduced until nearly a decade later.

The selective phenoxyacetic herbicides were developed not as a result of research aimed at assisting horticulture, but from a secret wartime project to develop compounds which could destroy rice fields and other vegetation and might thus be used as a weapon to starve enemy populations into submission. In 1943 Templeman and Sexton, working for Imeprial Chemical Industries in England, independently discovered the herbicidal activity of the phenoxyacetic acids. Such was the pace of discovery and development of synthetic pesticides that up to 6000 compunds have been patented out of which about 600 have found commercial use.

It is worth mentioning that the United States (US) perhaps tops the world's chart in the use of pesticides. So much was the use and abuse of pesticides in the US that Rachel Carson had to publish her historic book - "Silent Spring" in 1962 [4]. In the book she drew the attention of the government and people of the US to the dangers of the "biocides" they had embraced in the name of pest control. To some, "Silent Spring" sounded rather alarmist, but that was apparently the only way it could achieve its purpose. It marked the turning point in the use of pesticides the world over. It gave birth to the world interest in environmental

toxicology. Such was the controvery raised by "Silent Spring" that President Kennedy in 1963 appointed a special presidential task force to undertake a thorough appraisal of pesticides use in the US. The findings of the Advisory Committee resulted in the first legislation controlling the use of pesticides in the US. From that time the US became the most concerned country about the quality of her environment.

However, the publication of "Silent Spring" did not result in the expected decline in pesticide production and use. Since 1962, the total use of pesticides world-wide has more than trebled. More than 65% of all pesticides produced are used in North America, Western Europe and Japan. In 1974 all the developing countries combined used only about 10% of world pesticide production, and most of this was used in public health vector control programmes against flies and mosquitoes and on export cash crops. An FAO report in 1973 showed that, for 38 developing countries for which data were sufficiently detailed to allow analysis, pesticide consumption totalled 160,000 tonnes in terms of active ingredients. In 1975 the same 38 countries reported a total consumption of 202,000 tonnes.[5] This represents an annual increase of 21,000 tonnes

for the countries.

In Nigeria, the introduction of pesticides dates back to the colonial era when they were used mainly for the protection of export crops. In terms of distribution, more pesticides may have been used in the Western and Northern parts of the country than in the Eastern region. This is because cocoa and groundnut which were the main cash crops in the West and the North, respectively, are more susceptible to pest attack than the palm trees in the East.

In the IAR Farm the introduction of pesticides dates back to the 1950s when the farm was under the Agriculture Ministry of the defunct Northern Regional Government. Pesticides that were used in the farm include the organochlorines, organophosphates, and carbamates. However some of these are now being replaced by the synthetic pyrethroids because of the high effectiveness and low persistence of the latter.

Pesticide Distribution and Persistence in the Environment

Virtually everything on earth has been analyzed and found to contain pesticide residues. These include air, rain water, surface water, ground water, soil, foods, and tissues of plants and animals. In short, pesticide residues, especially those of organochlorines, have been described as

having become an intergral part of the biosphere.

In a study of the atmospheric transport of pesticides in different locations in the US, 0.003 ppm of dieldrin was found in the dust fallout collected in Cincinnati, Ohio.[6] In another study, dieldrin was found at a maximum concentration of 29.7 ng/m^3 in 50 out of 90 samples analyzed while aldrin was found in only one sample to a level of 8 ng/m^3.

The concentration of organochlorines in natural waters, expressed in terms of commercial formulations, has been found to be generally in the order of 1 ng/litre (1 pp 10^{12}) or less, except where locall pollution exists.[7] In the latter case, concentrations up to 1000 ng/litre may be detected, but often the value is 10 - 100 ng/litre. Analysis of many rivers in the US indicated that few exceeded 10 ng/litre of DDT or 5 ng/litre of DDE. In the predominantly unpolluted rivers of Scotland, the concentrations of DDT, DDE, and dieldrin was in the range of 1-3 ng/litre. In Japan, where BH_C was the most used pesticide up to 1971, marine waters contained BHC at levels up to 1 ppb while urban air contained 0.1-0.01 µg/m^3

DDT was found at levels up to 0.02 ppm in Mississippi at New Orleans. Fig. 1 is a proposed BHc distribution scheme and Tables 1 - 3 represent data on residue levels on some selected environments.

Pesticide Contamination of Surface Waters

Pollution of surface water by pesticides can be by either or both of two sources: intentional, and accidental means. Certain sources are quite apparent while others are not usually recognized. Pesticides residues are found in clean-up wastes from plants manufacturing active pesticide materials or formulating pesticide mixtures. One source often generally overlooked is the pesticide residues in the laundary waste water from the washing of protectives worn during manufacture or formulation. Wash water from drums which contained pesticides, and fireboard containers are potential sources of surface water contaminants.

Investigations from the IAR showed that there has never been and intentional treatment of the IAR Lake with any type of pesticide. There is also no record of dumping of industrial wastes or effluents into the lake. However, the villagers are known to do their laundary in the water and this may also apply to some labourers in the IAR Farms. Other possible

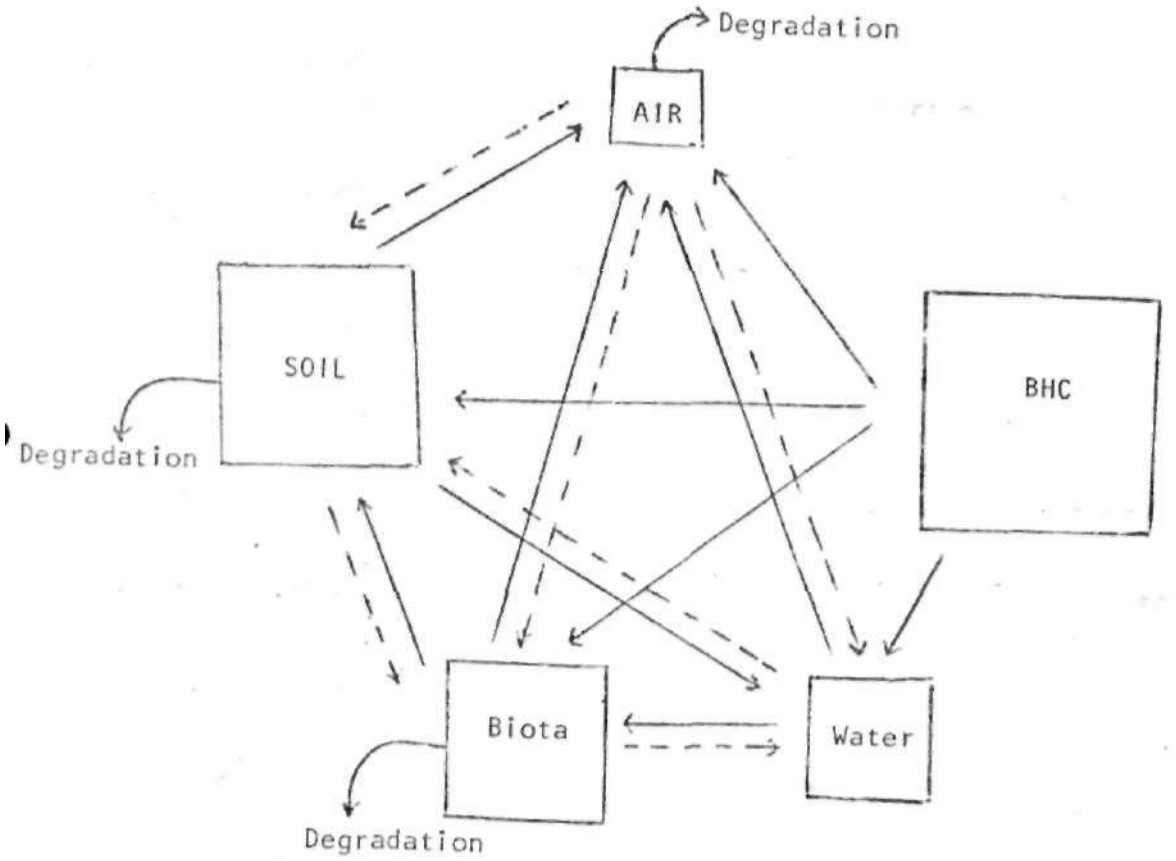


Fig. 1: Environmental distribution of BHC
(From Environmental Toxicology of Pesticides.
p. 238.

Table 1: Typical Amounts of DDT (ppm) in the environment.
(From Persistent Pesticides in The Environment p.1)

	ppm DDT		ppm DDT
Man	6.00	Aquatic invertebrates	0.10
Food vegetable	0.02	Plankton	0.05
Meat	0.20	Agricultural soil	2.00
Predatory mammals	1.00	Natural Soil	0.01
Predatory birds	10.00	Freshwater	0.00001
Herbivorous and insectivorous birds	2.00	Sea water	0.000001
Marine fish	0.50	Atmospheric dust	0.3
Herbivorous and Insectivorous mammals	0.50	Rain water	0.0005
		Air	0.0000000002
Insectivorous mammals	0.50	Aquatic plants	0.01
Freshwater fish	2.06		
Insects	1.00		
Plants	0.05		
Soil vertabrates	4.00		

Table 2: Distribution of Pesticides in lake California (1966). (From Persistent Pesticides in The Environment p. 32).

Attributes (ng/litre)	No of samples	DDT related compounds	BHC	Dieldrin	Hepta-chlor
Water (ppt)	82	0.60	0.01	T	T
Particle (ppt)	33	14.74	0	0	0
Bottom sediment (ppm)	39	4.44	T	T	T

T = Trace

Table 3 : Distribution of Pesticides in lakes (From Persistent Pesticides in the Environment p. 32)

Site	Total residues (DDT and related compounds (ppm))		
	Suspended particles (mg/kg)	Filterate (mg/kg)	Total water sample
Tule Lake	6.0	0.0004	0.00045
Lower Klamath	9.0	0.0004	0.00035
Deer Flat	12.5	0.0001	0.0004
McNavy	9.3	0.0001	0.0002

sources of chemical contamination of the lake are from car washing, and from empty cans including those use for pesticides. Additionally since much of the surrounding farmland drains into the lake, pesticides applied on those farms could ultimately end up in the lake.

Application

It is unavoidable that during the application of pesticide to a crop, a portion of the applied material will settle very slowly and be carried outside of the treated area by wind currents. If a stream is in the area of the drift, the water will be contaminated by the formulation. This may be more in the case of aerial application of the pesticides.

There has not been an aerial application of pesticides in the IAR, but it is possible that the drift can still occur even with the manual application methods used.

Surface Drainage

Surface drainage from treated croplands will contain pesticides in amounts ranging from picograms to micrograms per litre of water. Even when surface run-off of water does not occur, the down-ward movement of water-soluble pesticides through the soil and into the ground water will occur.

Erosion resulting from heavy rains or farm irrigation is a source of pesticide movement from treated to untreated areas and into surface waters. Adsorbed pesticides are carried with the soil particles, sometimes for considerable distances, and deposited in the beds of streams, in ponds, and lakes.

In the IAR Farms, there are three water channels which were constructed to direct runoff water and irrigation water from the farms to the IAR Lake. There is no erosion problem in the farms.

From all the above it is clear that the most important source of possible contamination of the IAR Lake is the agricultural processes on the nearby farmlands.

Pesticide persistence has been studied extensively in water and soil. The persistence of pesticides is normally studies following single exposures, as repeated treatments o obscure the actual rate. However, since most treatments are repeated, it is sometimes necessary to estimate rather crudely what the total amounts applied have been and then make annual assessment of residual amounts from which an approximate rate of disappearance can be made.

Soil

DDT, BHC, chlordan, dieldrin and heptachlor last for long periods in soils. Plots treated experimentally with the unusually high amount of 100 lbs of DDT per acre in 1947 had residues of 28.2 lb per acre in 1951. This is an average yearly loss of about 18% of the total or about 27% of the residue remaining for each year.

In another experiment, BHc applied at 100 lbs per acre was reduced by about half after 3 years. This represents an annual rate of disapperance of about 20%. Flemming and Maines (1953) reported a loss of about 10% per year over 8 years in areas heavily treated with DDT for Japenese Beetle.[8] Litchenstein (1957), in a study involving 14 orchards, reported a recovery of 26.6% of the total amount of DDT applied as spray for a ten-year period.[9] Other researchers have placed the respective annual losses of DDT and BHC at about 12 and 16%.

Lindane and aldrin residues in loam soils did not s survive as well. After two years for the former and one half years for the latter only 10 and 13% respectively, were detectable. Heptachlor was not recovered from soils after 9 years of treatment, but the conversion product - heptachlor epoxide - was present at about 5% of the original

application rate. In another study, 12% of a single application of granular heptachlor was recoverable in soil after 6 months. Tables 4 - 6 show the persistence of different pesticides in a variety of soils types.

Water

In discussing the persistence of organochlorines in water, reference is often made to the experiment by Hamelink et al (1972).[6] They used a flooded rectangular limestone quarry as a model ecosystem. The quarry was stocked with rainhow trout and subsequently treated with a single 0.05 µg/ L (ppb) dose of both lindane and DDE. Residue levels were then measured in this and adjacent lakes over a period of 3 years.

Analysis of the water showed that DDE concentration stabilized at near 1 ppt after 120 days while lindane levels declined from 25 ppt on day 123 to 13 ppt on day 258. Hence only 1 or 2% of the DDE persisted in the water for any appreciable length of time while lindane had a half-life of about 120 - 150 days. A high concentration (24.21 ± 10.58 ppb) of DDE was found in the top 15 cm layer bottom mud on day 123. Significant levels of residues of both pesticides were still found in the biota (aquatic plants and animals) which had accumulated the residues to levels

Table 4: DDT recovered in 1955 from upper 15 cm of silty clay loam turf plots in Cleveland, Ohio, treated in 1945 only.[9]

DDT applied (lb/acre)	DDT Recovered		
	ppm	lb /acre	% amount applied
12.5	0.77	1.36	10.9
25.0	2.04	3.52	14.1
37.5	3.89	6.72	17.9

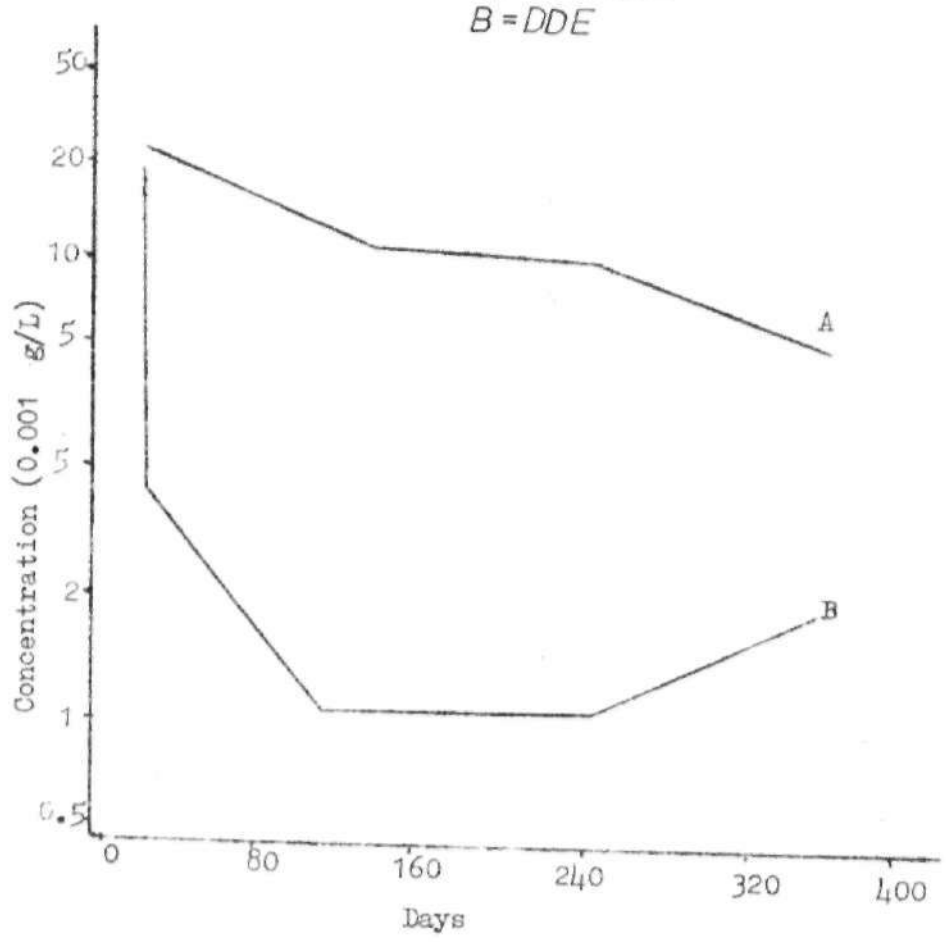
Table 5: Average residual DDT in 85 soils treated with DDT at the rate of 25lb/3inch acre.[8]

After indicated years	Percentage (%)
1	97
2	90
3	79
4	64
6	56
8	44

Table 6: Persistence of DDT in soils of different textures[8]

Texture	No. of Sites	Lbs/acre after indicated months						
		0-4	12-16	24-28	36-40	48-52	72-76	96-100
Sand	3	25	24.8	23.3	22.0	21.0	20.8	18.5
Stony Loam	6	25	24.8	22.3	18.6	14.6	12.0	10.0
Sandy Loam	37	25	24.6	22.9	20.4	16.8	14.9	12.9
Loam	21	25	24.0	21.9	18.7	14.8	13.5	9.4
Silt loam	12	25	24.9	23.9	21.1	16.7	11.7	8.5
Clay loam	5	25	23.4	21.4	18.2	-	-	-
Muck	1	23	14.0	9.5	6.0	3.0	-	-

Fig. 2: The concentrations of residual DDE and Lindane in the water versus time. (From the Fate of Organic Pollutants in the Air and Water Environments p. 267.)

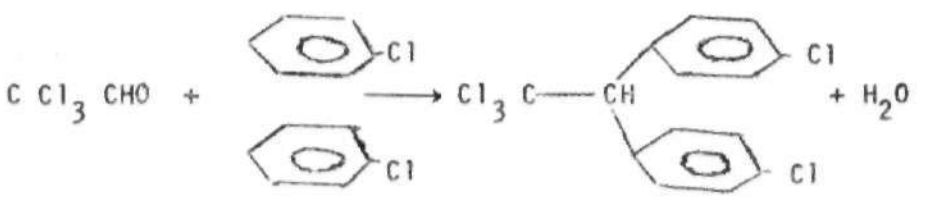


hundreds of times above that in the water. Fig. 2 shows a graph of the decline of the residue levels in water with time.

1.3 The Chemistry of DDT, BHC And Endosulfan

DDT

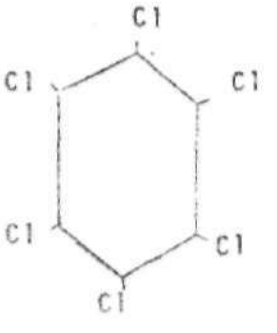
Dichlorodiphenyltrichloroethane (DDT) is a white amorphous powder produced by reacting chloral with chlorobenzene in the presence of sulphuric acid.



Chloral monochlorobenzene DDT

DDT exists as two isomers: o,p^1 - and p,p^1 DDT, with the p,p^1 isomer being more toxic. DDT has a molecular weight of 354.5, and the pure substance melts at 108-9°C, DDT contains 11% hydrolyzable chlorine and 1% volatile and alcohol - insoluble materials. It can be formulated as solutions, emulsifiable concentrates, dusting powder, wettable powder, aerosols, and insecticidal smokes.

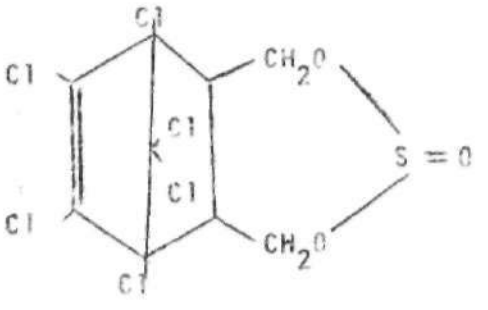
BHC



Benzene Hexachloride (BHC) is a greyish to brownish amorphous solid with a characteristics musty odour. It is prepared by the chlorination of benzene in the presence of UV e.g. sunlight. The crude product consists of six chemically distinct isomers with the γ isomer having the most insecticidal property.[10] Lindane has been adopted as the name for the purified product containing 99% or higher of the γ isomer.

BHC has a molecular weight of 290.9 and melts at 112°C. Lindane is formulated as wettable powders, solutions, aerosols, and emulsifiable concentates.

Endosulfan



Endosulfan belongs to the diene-organochlorines or cyclodiene chlorinated insecticides. It can be prepared by the Diene-synthesis or Diels-Alder reaction, i.e. the addition of a compound containing a double or a triple bond to the 1,4 - positions of a conjugated system.[1]

Endosulfan has a molecular weight of 406.95. The technical product = cream to brown cloured flakes -, - consists of a mixture of α and β isomers with melting points of 108-110°C and 208-210°C respectively. Endosulfan is stable when stored under normal conditions but susceptible to slow hydrolysis by moisture, aqeous alkali and acids. It is formulated as wettable powders, emulsiable concentrates and dispersible powders.

1.4 The Toxicity of Pesticides

Pesticide toxicity can be considered under two broad categories: the immediate and long-term effects. The immediate effect is by an acute disturbance of the nervous system which, if severe enough to interfere with respiration, may be fatal. The long-term effects of pesticides are not quite understood but many speculations have been made, based on symptoms observed in treated laboratory animals. Dieldrin fed at levels as low as 2.5 ppm in the diet shortened the life time of mice, and was an inducer of liver hepatomas in male mice at the low dosage

of 0.1 ppm. Other studies have shown that aldrin, dieldrin, DDT, mirex, strobane, heptachlor, and chlolabenzilates are positive for tumour induction in human beings.[11] It has also been found that if a woman was poisoned by an insecticide and subsequently found to be pregnant, therapeutic abortion would be envisaged.

Pesticide poisoning affects the drug metabolism of patients, and the fertility of animals. Racoons fed on a diet containing 2 ppm of dieldrin had their estrus later than did the controls.

Birds are particularly vulnerable to organochlorine poisoning. In a laboratory study, American kestrels were maintained on a diet containing 2.8 ppm p,p' DDT and their egg shells were observed to be 10% thinner than those of the controls after 2 years. In another study, the animals were fed on diets containing 0.84 ppm dieldrin and 4.7 ppm DDT and the result was high reproductive failures due to increased egg disappearance and breakage and egg eating by adults. Organochlorines enhance the metabolism of steriods which control the deposition of medullary bone. The medullary bone is the main source of calcium during egg shell formation, hence the egg shell thinning.[12]

There are many reports of heavy fish killings resulting from pesticide poisoning. 8 ppm of DDT in the ovaries of spotted seatrout has been reported to result in loss of spawning, while lake trout eggs containing the same amount of DDT resulted in the death of all newly hatched fish. (See fish toxicity data in Table 7).

1.5 Review of Residue Analytical Techniques And Instrumentation

There is a large number of references on the analytical methods and instruments that have been used for pesticides residue analyses.[13] The procedures differ, depending on the source and type of the analytic samples,[14] the expected level of residues, and the degree of accuracy required. (See Fig. 3). There are however three basic steps in each method - extraction, cleanup, and determination.[15]

Extraction

Many organic solvents and their mixtures are widely used for the extraction of residues from many sources. The solvent of choice depends on the nature of the sample, the polarity of the residues, and the method to be used for the determination of the residues. Acetonitrile is very useful for extraction in the presence of fat[16] and is therefore the solvent

Fig. 3: Scheme for the extraction and cleanup of residues from different environments. (From Environmental Toxicology of Pesticides p. 237).

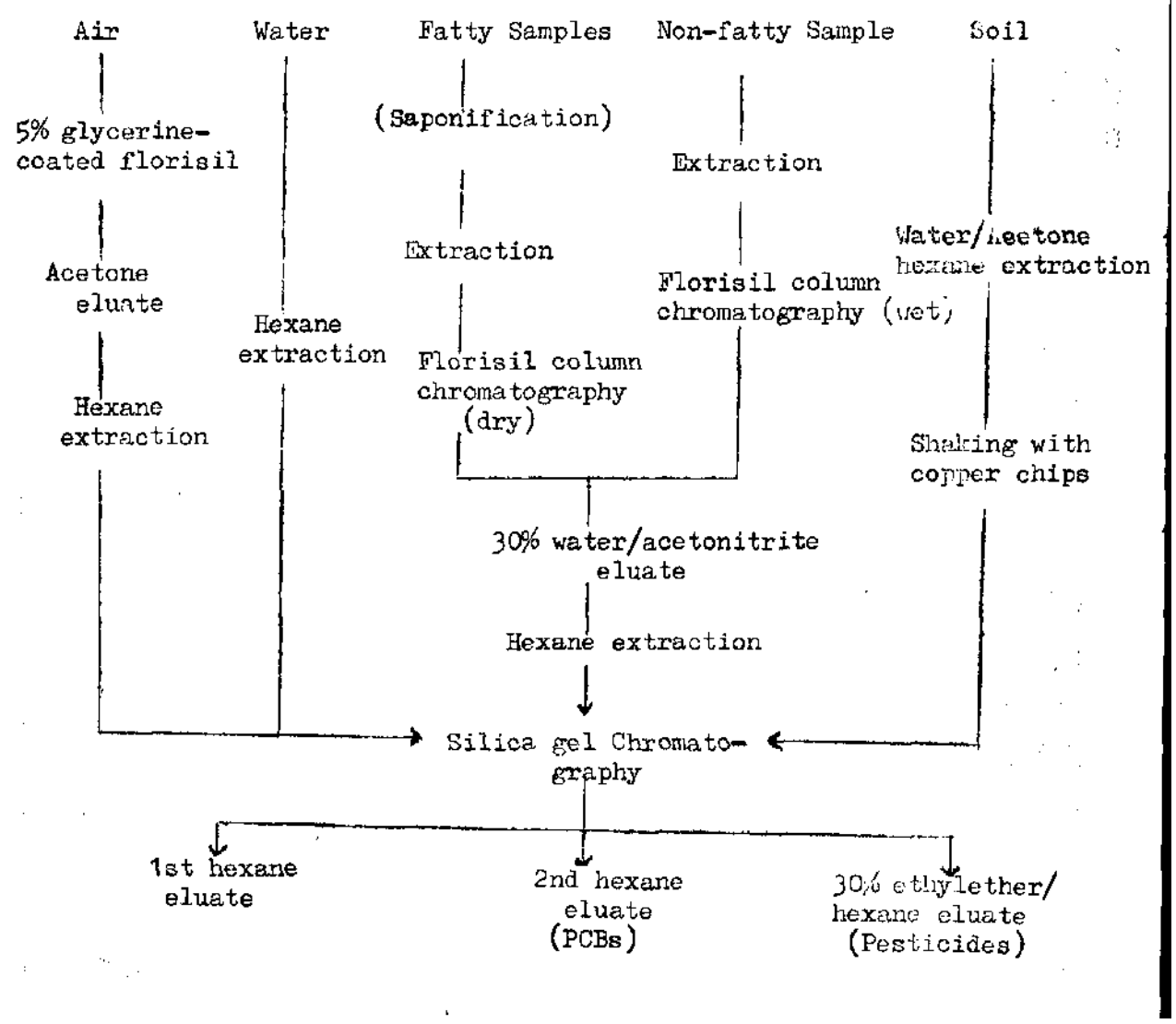


Table 7: Lethal limits of organochlorine insecticides to fish. (From Persistent Pesticides in the Environment p. 66).

Insecticide	Fish Tested	Concentration (ppm)	Exposure time (hr.)
DDT	Bluegill	0.016	96
	Salmon	0.08	36
	Goldfish	0.27	96
	Rainbow trout	0.002	96
Lindane	Bluegill	0.077	96
	Goldfish	0.152	96
	Guppy	0.138	96
BHC	Bluegill	0.79	96
	Goldfish	2.30	96
	Guppy	2.17	96
	Rainbow trout	0.22	96
Aldrin	Bluegill	0.013	96
	Goldfish	0.28	96
	Guppy	0.033	96
	Rainbow trout	0.031	96

of choice when residues are to be extracted from plant, animal,or food materials. If the residues are going to be determined by thin-layer chromatography, or gas chromatography with the use of electron capture detector or with microcoulometric detector, highly volatile hydrocarbons e.g. pet ether or isooctane are preferred. For IR-spectrophotometric determination, carbon tetrachloride, which is transparent in the important spectral regions, is often employed.

For the extraction of residues from water,[17,18,19] the carbon adsorption technique is very popular. One method is to run the water through a charcoal adsorptive cartridge.[20] The charcoal in the cartridge is then exposed and air-dried at 40°C for 48 hrs. Chloroform is used to extract the residues in a Soxhlet extractor. The extraction is done for 35 hrs at the syphoning rate of 2-cylinder volumes per hour. The chloroform extract is evaporated to dryness and the carbon-chloroform extract (CCE) is obtained. If polar residues are of interest, the choroform- extracted carbon is re-extracted with ethanol for 24 hrs. and a carbon-ethanol extract (CAE) is obtained.

In another procedure, a carbon adsorption column[21] is used. A chromatographic column can be used for this purpose. The column is plugged with pre-extracted glass wool at the lower end and filled with deionized water. Activated charcoal is slowly added to the column and any floating carbon dust is decanted off. The top layer of the settled carbon is covered with glass wool, and the water is drained off. The sample is then passed through the column. It is believed that 1 gm of charcoal can remove all the residues in up to 5L of contaminated water. After the adsorption the carbon is extruded, and the adsorbed residues are extracted with appropriate solvents.

Residues in liquid samples can also be extracted by liquid - liquid extraction. In this technique the p-values of the pesticides in different solvents is exploited. The extraction can be done semi-automatically, or with separatory funnels. Solid samples may be extracted by liquid partioning using Soxhlet extractors.

Cleanup

This is very useful for the removal of impurities and interfering substances, and for the resolution of the residues into different fractions to simplify their determination. Cleanup can be achieved by liquid partitioning but the use of chromatography is more popular. Thin-

layer,gas-liquid, and column chromatographic's have been extensively used. Adsorbents[22] used include silica gel, alumina, and florisil in the activated or deactivated state.

Determination

Residue determination can be divided into three basic areas of measurement - biological, chemical and physical. Undoubtedly, the physical methods of measurement dominate the present residue analytical field. These include the chromatographic and spectrometric techniques. The next is chemical methods. Bioassays appear to be fading out of the pesticide residue picture, except perhaps as a parallel screening technique.[23]

Chromatographic Methods

The application of gas chromatography to the quantititative determination of pesticides, and as an aid in their qualitative identification, has flourished in the past two decades.[24] Gas chromatography has been found very useful in the resolution and quantitiation of thiodan isomers;[25] and for the analysis of the organochlorines in a mixture.[26] Residue analysis can also be effected with a combination of gas chromatography and infrared spectrophotometry. Gunter et al (1960)[27] reported the application of this technique for the determination of

chlorinated pesticides.

The detectors commonly in use in the gas chromatographs for pesticide analysis include the ionization detector, the microcoulometric detector, and the electron capture detector (ECD).

Flame Ionization Detectors

The detectors under this group were developed after the discovery that the addition of sodium salt to the hydrogen-oxygen flame of a flame ionization detector enhanced the ionization current produced when halogen - and phosphorus - containing compunds entered the flame.

Microcoulometric Detectors

These monitor the electrical changes caused when reactive substances eluted by the carrier gas enter a specially designed titration cell. It affords a highly selective detection system, and is good for the detection of chlorine - , bromine - , iodine -, sulphur -, phosphorus-, and nitrogen - containing organic compounds.

Chlorine -, and sulphur - containing pesticide residues can be determined by the continuous titration of the ions with silver ions that are electrically generated in the titration cell.[28]

Electron Capture Detector (ECD)

The ECD monitors the decrease in cell current which occurs when electronegative species absorb slow electrons. The slow electrons are produced as a result of the ionization of the carrier gas by a radioactive source. This is a highly sensitive detector, but its non-specific nature is often a source of problems when unknowns are analyzed

A comparative review of the various gas chromatographic techniques showed that the results obtained using the ECD and the microcoulometric detectors were better in terms of sensitivity.[29]

Colorimetry, which is a relatively old method, still finds some use in the analysis of specific residues. The technique consists of saponification or derivatization of the residues to obtain specific colours which are determined spectrophotometrically.[21,30-33]

Other methods of less importance include bioassay, phosphorimetry, polarography, and infrared spectroscopy.[34-36] In general, methods like bioassay and colorimetry involve elaborate cleanup procedures if they are to be free from interferences, and are laborious and time-consuming. In infrared spectroscopy, the inherent difficulty of interpretation of the spectra presents a problem. The method is also not good for quantitation.

These problems with the other methods make gas chromotographic techniques the most versatile methods used to date. Their advantages include high sensitivity and the very small sample needed for the analysis. Gas chromatography was therefore chosen for this work.

1.6 Research Objective

Efforts have been made by many governments in Nigeria to diversify the economy of the country from the present almost total dependence on petroleum. This led in 1976 to the launching of the Operation Feed the Nation (OFN) and, later the Green Revolution; and also to the building of iron and steel factory and steel rolling mills in different parts of the country. One consequence of the emphasis on an agro-based economy is the increased usage of agro-chemicals (pesticides and fertilizers) in all parts of the country. This is sometimes without the necessary thorough screening and expertise that would ensure the effective use and safe handling of these "poisons". Pesticides, including those whose use have been banned in other parts of the world for reasons of safety, are known to still be in use in the country.

The aim of this research is to determine the occurrence and level oforganochlorines in one part of our environment - surface water (IAR Lake). The knowledge of this will enable us estimate, directly or through subsequent researches, the amount of residues in our aquatic life forms e.g. fishes and aquatic plants, and on the crops and grasses irrigated with water from the lake, the animals that feed on these grasses and also drink the lake water, and ultimately on man who dependson these plants and animals for his food.

The result obtained for the IAR Lake will be compared to those for polluted and unpolluted waters in different parts of the world. Based on the recommended upper limits of the various residues in surface water, a decision would then be made on whether the lake can safely be used for the various purposes which it currently serves.

CHAPTER 2

SAMPLING

2.1 Sampling Theory

The importance of good sampling in analytical work cannot be over-emphasized. All the careful work put into a good analysis will go for naught if the sample taken is not representative of the material to be determined. If the material to be analyzed is homogeneous, sampling does not create a problem. Formidable problems are likely to arise when the material is heterogeneous or is otherwise non-uniform.

Stratified Sampling[37,38]

A sampling process is called Stratified if the entire population is divided into mutually exclusive groups and some samples are randomly selected from each group. Lakes have more often than not been found to be stratified and because samples for this research will be obtained from lake water, it is considered necessary to briefly describe the principles underlying the stratified sampling technique.

Stratification of a population is said to occur when there is a wide variation in the overall population characteristics, but there are possible groupings within the

population within which the variation is low. The groupings are referred to as strata.

Strata Sample Sizes

Stratum sample size will need to be proportional to the stratum size, proportional to the stratum standard deviation, and inversely proportional to the square root of the unit sampling cost in the stratum. Hence, large, highly variable strata with low unit sampling costs will lead to large samples relative to other strata.

Proportional allocation is used, if information on stratum variances and sampling costs are not available.

Different patterns of stratification may exist in the IAR Lake with reference to pesticide residue content due to a variety of factors.

(a) Sedimentation Stratification

Due to the low solubility in water of many pesticides, residues adsorbed to suspended particles in the water may tend to settle, thereby giving rise to horizontal stratification of the water.

Visual observation showed that the IAR Lake contained suspended particles but the presence of horizontal stratification could not be confirmed.

(b) Stratification Due to Differential Chemical Reactions

A stratification similar to that described above can also arise from differences in rates of chemical reactions taking place at different depths in the water. Some of these reactions, e.g. photoxidation or photo-degradation would be expected to be higher on the surface than at other depths thus giving rise to horizontal stratification.

(c) Stratification Due to Differential Biological Activities

It is known that by a process of bioaccumulation, some plants and animals are able to accumulate pesticide residues in their systems to a level far above that in their environments. If this happens it will result in the depletion of the pesticides in their surrounding.[6] This is a possible cause of stratification in the IAR Lake because about $\frac{1}{4}$ of the total lake surface was covered by water lilies, about $\frac{1}{2}$ was covered by grass, and the remaining area was open surface at the time the samples were taken.

(d) Stratification Due to The Location of Farms and Inflows

As the expected residues in the lake are supposed to arise from the activities on the nearby farms, the location and distance of the surrounding farms is crucial. Also important is the direction of inflow of farm runoff water into

the lake. These can give rise to a stratification of the lake.

The southern part of the IAR Lake is bordered by the IAR Farms, the western end was dammed, and the remaining sides are near the villagers' farms. From the IAR Farms,three water channels direct runoff water into the lake. More residue would therefore be expected to be reaching the lake from the side bordering the IAR Farms, and little from the villagers' farms and almost none from the d dammed end.

2.2 Sampling Techniques

There is no general procedure for establishing a satisfactory sampling programme applicable to all situations. A few samples may suffice in some cases while, in other cases, frequent sampling at many locations may be necessary. No matter which method is chosen, the aim is to get sample statistics that can be applied with great confidence to the population under study. To achieve this it is necessary to:

a) obtain a sample whose concentration of the determinands are identical to those in the bulk of the substance of interest at the time of sampling;

b) repeat this sampling operation at time such that the required information of any changes in water quality is obtained;

c) ensure that the concentrations of the determinands in the samples do not change between sampling and analysis.

Types of Samples

There are six major types of samples classified by their method of collection:

a) Individual grab sample

b) Simple composite

c) Sequential composite

d) Continuous sample

e) Hand proportioned composite

f) Automatically proportioned composite

Individual grab sample is got when the sample is retained as a separate entity in its own container.

Simple composite requires that all samples taken over a specified time interval be deposited in a single container.

Sequential composite is the collection of a series of individual samples per container, each container representing a specified time period. This is particularly useful where the characteristic of the substance being determined may vary significantly from hour to hour. In a continuous sample a small amount is collected in continuous flow. It is useful in pilot scale processes. The method is usually not used in cases of high suspended solids unless provision is made to avoid settling on line pluggage.

The hand proportioned sample is readily obtainable where flow charts are available. Individual or sequential composite samples are manually composited in proportion to flow in order to obtain this representative type sample.

The automatically proportioned composite requires additional equipment. Sampling in proportion to flow is achieved by varying the size of individual aliquots in proportion to flow volumes.

Sampling Sites

Generally, sampling from the boundaries of water systems should be avoided unless these regions are of direct interest.[39] The concentration of determinands at these points

are often not representative of the bulk of the water. The locations that should be avoided include, the banks, surfaces or bottoms of water systems. It should be noted that lakes can be stratified vertically but be homogeneous horizontally.

Inhomogeneous distribution of determinands in water may arise from a variety of factors.

Undissolved materials tend to distribute themselves heterogeneously if their densities differ from that of the water. Inorganic sediments in natural waters have been found to increase in concentration with increasing depth because the particles tend to settle, while certain biological detritus and oil tend to rise to the surface.

Chemical and or biological reactions may occur to different extents in different parts of the water of interest, and thus lead to heterogeneity of substances involved in these reactions. These reactions are often confined to the boundaries of the system of interest e.g. banks, beds, or surfaces of the water. Sampling in these regions should therefore be avoided.

If there is no obvious indication of layers of immiscible liquids, or settled suspended matter, a composite sample can be taken by lowering a jar or glass cylinder at a steady rate to the bottom of the water system without stopping, and raising it to the top at the same rate. The rate should be such that the jar is just filled as it reaches the top surface of the water.

Another method of sampling relatively homogeneous water systems is to collect samples of the same volume from the top, middle and bottom portions of the water.

One technique that has been used for residue sampling is to suspend sheets of polyethylene in the water for some days. These are then extracted with suitable solvents. This technique is based on the principle that pesticides tend to get adsorbed on polyethylene and plastic containers.

To reduce the number of samples, and hence the number of analyses, it is good to get a composite sample by merging many samples. But the accuracy of the estimate of average quality will be worse than that obtained from analysis of a number of discrete samples if this accuracy is governed by random analytical errors rather than by variations in quality.

Samplers

A variety of instruments have been used for water sampling depending on the adaptation of these to local situations. Below are some of the many that have been used.

From boat - a Golden Thief hand vacuum pump which can lift from depths of 25 ft.

From Bridge - a weighted pail tied to a rope

From Standing water (Rice Paddy) - a glass tumbler - representative sampling.

From Runoff water - a metal pail.

To avoid the problems of interaction between determinands and container materials, and to minimize the difficulty in extraction due to adsorption of pesticides to sample containers, glass material should where possible, be used in collecting samples for pesticide analysis.

In this work, a sports boat was utilized to collect samples from the lake. The sampler was an oversized chromatographic tube (5 cm id x 141 cm) with a teflon stopper. To collect a sample, the stopper is opened and the tube is vertically dipped into the water with the open end. When the tube is filled with water to the required level, the stopper

is closed and the tube, with the column of water, is raised. When the open end gets to near the surface of the water it is closed with the palm and the tube is turned upside-down. The water is then transferred into the sample bottle through the stopper.

Sample preservation and Storage

Water samples collected for residue analysis can be preserved by acidifying to pH4-pH5 with appropriate dilute acid. Samples are stored in chemically clean glass bottles closed with a screw cap with aluminium foil insert. The bottles should be completely filled with the water and stored away from light in a cool place until required for analysis.

2.3 The IAR Farm and Lake

The IAR Farm is located on the Samaru-Funtua road at a distance of about 3 km from Samaru. It was established by the defunct Northern Regional Government but later transferred to Ahmadu Bello University which now uses it for teaching and for research. The farm is divided into plots which are alloted to the Institutes different departments which plant their respective plots with a variety of crops. The main crops planted include sorghum, millet, wheat, barley,cowpea, and soybeans. Others are groundnut, sesame,

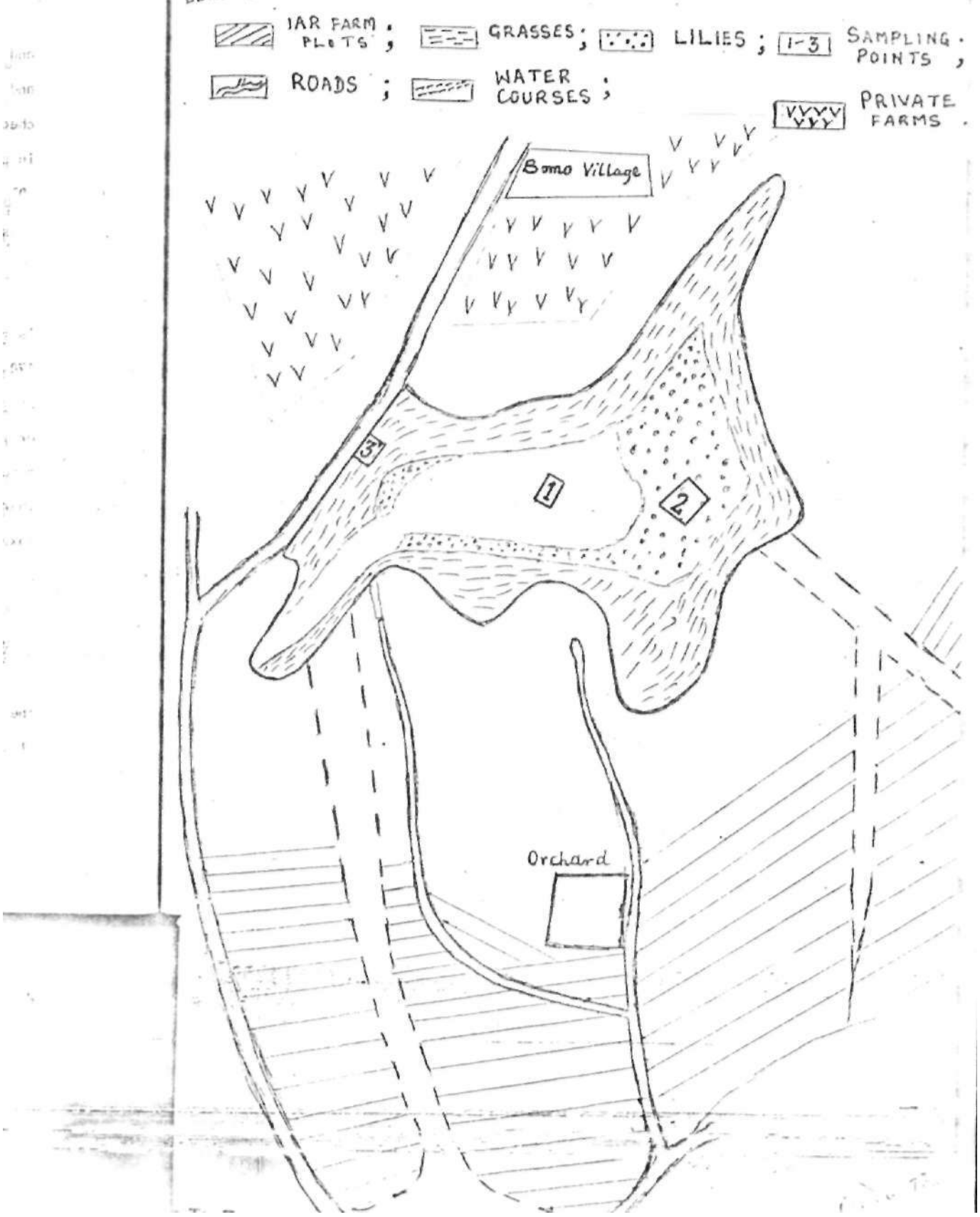
and other economically important oil seeds, as well as cotton and other fibres. The farm is cultivated annually and, to check the menace of pests, a number of pesticides have been in use in the farm since its establishment. .These include DDT, BHC, endosulfan, aldrin - dieldrin, some organo-phosphates and carbamates, and more recently the synthetic pyrethroids.

Near the IAR Farm, and adjacent to the Bomo village, is the IAR Lake (Fig. 4). The lake covers an area of about 170,000 m^2 and serves as part of the reservoir for the university irrigation scheme. Runoff water from these farms drains back into the Lake. About $\frac{1}{4}$ of the lake surface is covered by lilies, $\frac{1}{2}$ by grass and the rest is an open surface. There are three prominent water channels leading into the lake from the IAR Farms.

A variety of small fishes is known to abound in this lake. The villagers and IAR Farm labourers use the lake water for their laundry and for their livestock.

One sample each was taken from the lily-covered region, the grassy portion, and the uncovered section. The state of the lake on the day the camples were collected were :

FIG. 4: THE IAR-LAKE AND FARM, SAMARU - ZARIA.



as follows:

Average depth - ... 2.25 m

Temperature of the lake water .. 15°C

Current - - - - - a gentle surface current caused by the prevailing north-east wind.

The water depth was measured at selected points with a graduated conduit pipe. The temperature of the water was taken at the surface with a thermometer.

The samples were collected between 10 and 11 a.m. from the locations shown in Fig. 4. The sample from the uncovered region was labelled SI; those from the lily-covered region and the grassy portion were labelled SII and SIII, respectively.

CHAPTER 3

PRELIMINARY ANALYSES

3.1 The Determination of Optimal GLC Analytical Conditions for BHC, DDT and Endosulfan

In recognition of the numerous possibilities that exist in GLC a series of experiments was carried out to determine the suitable conditions for the analysis of BHC, DDT, Carbaryl, and endosulfan using the Varian Model 3700 gas chromatograph. The conditions under consideration include:

i. column stationary and mobile phases,
ii. detector types
iii. injector temperature
iv. column temperature,
v. detector temperature,
vi. and chart speed
vii. Gas flow rate
viii. column head pressure
ix. Attenuation

The objective was to establish operating conditions that give narrow and well-resolved peaks. To achieve this, some established criteria were borne in mind. For example, account was taken of the recommended maximum temperatures and other properties of the common stationary phases in the adjustment of the chromatographic parameters.

The Varian Model 3700 gas chromatograph that was used for this work had the following accessories:

a) Digital temperature controls for the injectors, column oven and detectors.

b) 5'x ¼" stainless steel column packed with 10% carbowax 20 M on Chrom W support (Column A)

c) 5' x ⅛" stainless steel column packed with OV - 1 on Chrom W support (Column B).

d) Column temperature programmer.

e) Thermal conductivity detector (TCD)

f) Flame ionization detector (FID)

g) Electron capture detector (ECD) with ^{63}Ni as the electron source.

A 1% (w/v) solution of each of the above standard pesticides was prepared in n-hexane, except carbaryl which was prepared in methanol.

The first column tried was column A and the operating conditions were set as below:

Injector temperature	.....	250°C
Column temperature	.....	200°C
Detector "	.....	300°C

Column pressure	.....	10 PSIG
Nitrogen carrier gas flow rate		25 ml/min.
Detector	.....	FID
Chart speed	.....	2min/cm.

Under these conditions, none of the samples injected was detected after 12 mins. On programming the column temperature from 160 to 200^{0}C, the pesticides were detected as irregular tailed peaks, and the technical DDT was not resolved into the expected isomers.

The experiment was continued using column B. Using the FID, the operating conditions were adjusted until the expected peak shapes, resolutions, as well as consistent retention times were obtained. These were got under the following conditions

Injector temperature	.....	220^{O}C
Column "	.....	200^{O}C
Detector "	.....	250^{O}C
Column pressure	.....	10 PSIG
Gas flow rate	.....	30 ml/min
Chart speed	.....	2 min/cm
attenuation	.....	64

The retention times (in seconds) of the different pesticides were as follows:

Pesticides	Retention Time (secs,)
Carbaryl	72
γ BHC	96
α- endosulfan	336
β- endosulfan	480
0, p DDT	540
p,p' DDT	696

To study possible interferences, a mixture of all the pesticides was injected into the column. Only the endosulfan and o, p' DDT peaks coincided. All the others were resolved.

After determining the retention times from the above experiment, the column was purged for 24 hours and the FID was replaced with the ECD. There was a change in the retention times as a result of the purging. The ECD proved to have higher sensitivity for all the pesticides than the FID. The new retention times (secs.) were:

Pesticides	Retention Time (secs.)
Carbaryl	60
ɣ BHC	108
∝- endosulfan	342
β- endosulfan	540
o,p' DDT	408
p,p' DDT	510

From the above experiments, it was concluded that Column B was better for the analysis of the pesticides than Clumn A, and that ECD gives a higher sensitivity under the conditions as established above. The above conditions and equipments were therefore left unchanged for the subsequent analyses.

3.2 Preparation of Calibration Curves

Serial dilution (ppm w/v) of ɣ BHC, o,p'DDT, p,p' DDT ∝-endosulfan, and β-endosulfan were prepared in n-hexane. For injection into the gas chromatograph, 1µl aliquots were taken from the different dilutions. The quantities of the pesticides injected was calculated in terms of the amount in nanograms of the substance in the injected volume.

For example 1µl of 1 ppm solution = 1 ng solute, 1 µl of 2 ppm solution = 2 ng solute etc. The response by the detector was measured in terms of peak height in milimeter. Two injections were made for each dilution and the average peak heights were used for the plots. Figs 5-8 show that the plot of peak height versus the amount of pesticide injected were linear for all the pesticides at very low quantities of pesticides. The minium detectable quantity (MDQ) was determined from the calibration curves as the amount of pesticides to give a response equivalent to twice the background noise. The values for the different pesticides are as below:

0.15 ng for γ BHC

0.45 ng for o,p' DDT

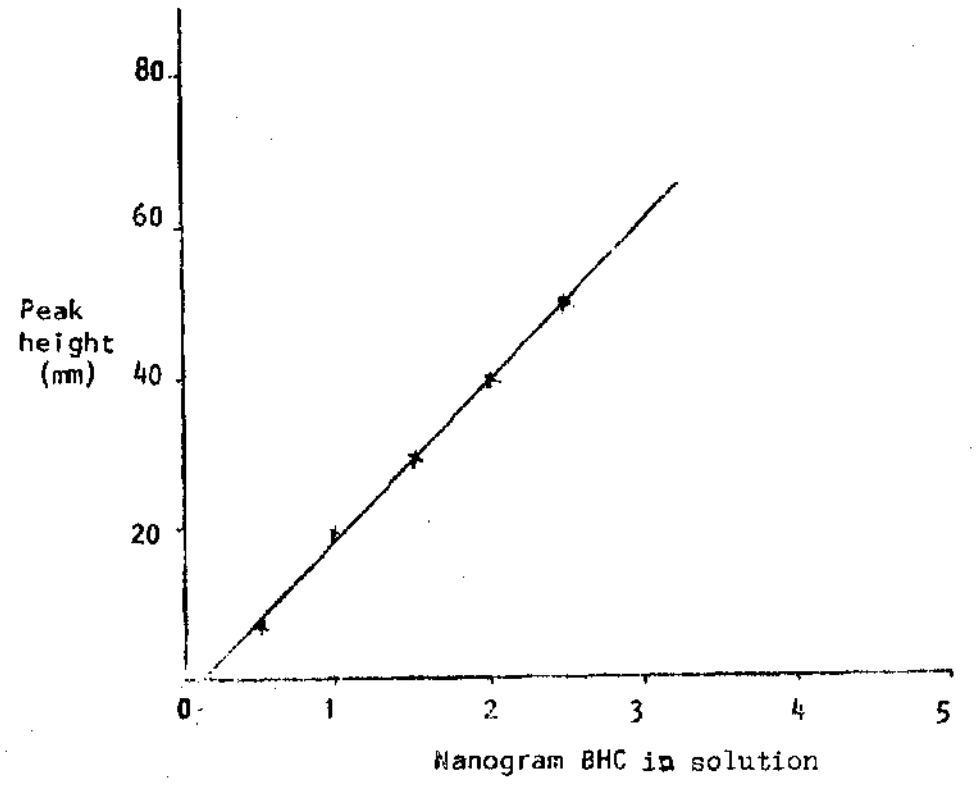
0.40 ng for p,p' DDT

0.015 ng for α-endosulfan

0.50 ng for β-endosulfan

Fig. 5 Calibration Curve for ɣBHC

Quantity of ɣBHC(ng)	0.5	1.0	1.5	2.0	2.5	5.0
Peak heing height (mm)	7	20	30	40	50	65



Calibration Curves for o,p' DDT & p,p' DDT.

Quantity of o,p' DDT (ng)	10	15	17.5	20	21.5	25
Peak height (mm)	8	14	19	22	24	31

Quantity of p,p' DDT (ng)	10	15	17.5	20	21.5	25
Peak height (mm)	26	41	49	52	56	65

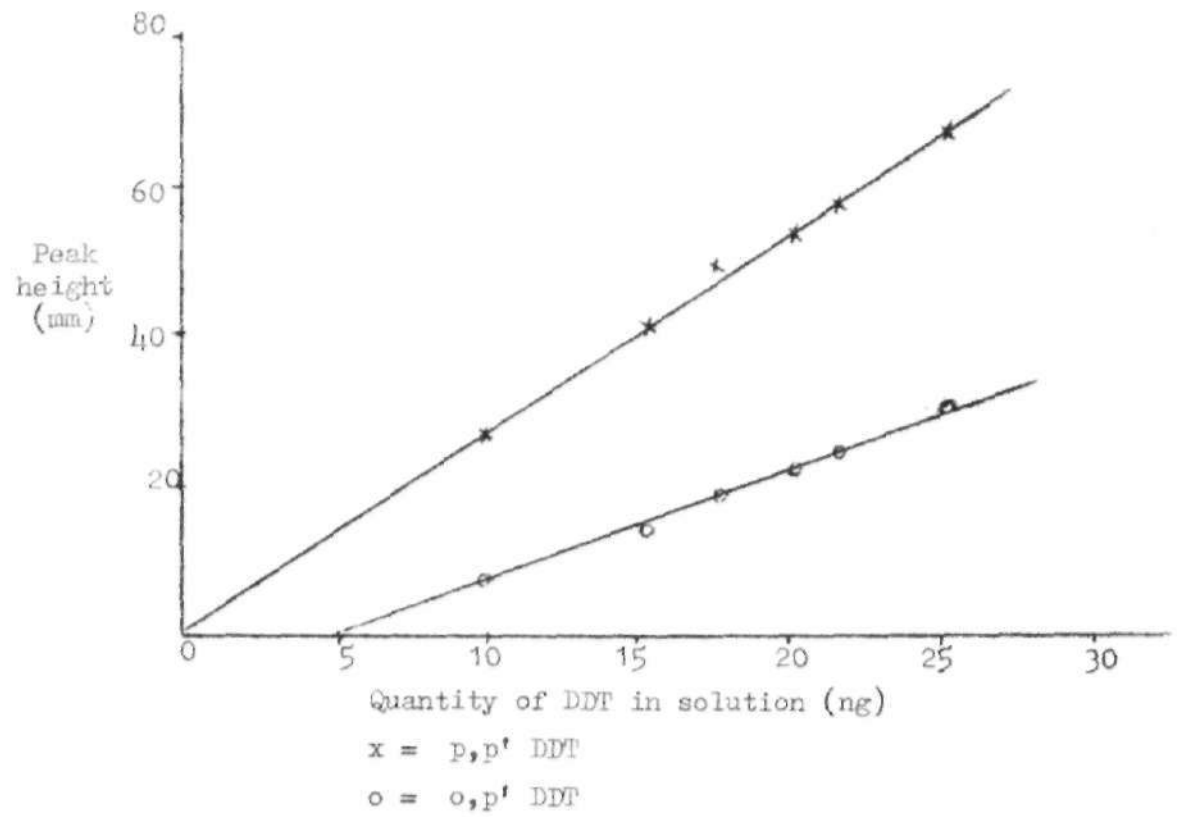


Fig. 7 Calibration Curve for ∠-Endosulfan

Quantity of ∠-endosulfan(ng)	0.1	0.2	0.3	0.5	1.0	1.5
Peak height (mm)	3.0	4.0	5.0	9.0	12.0	17.0

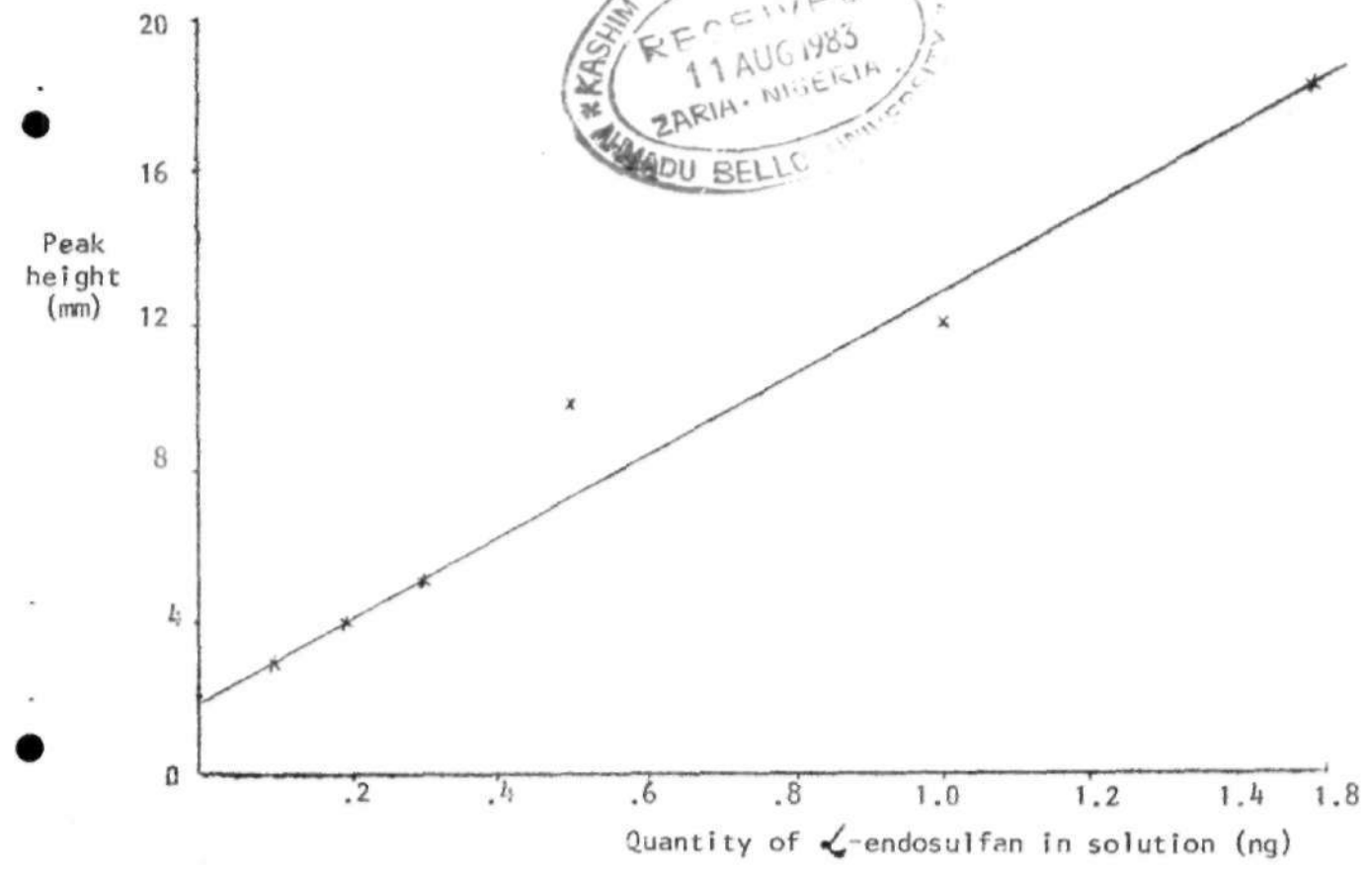
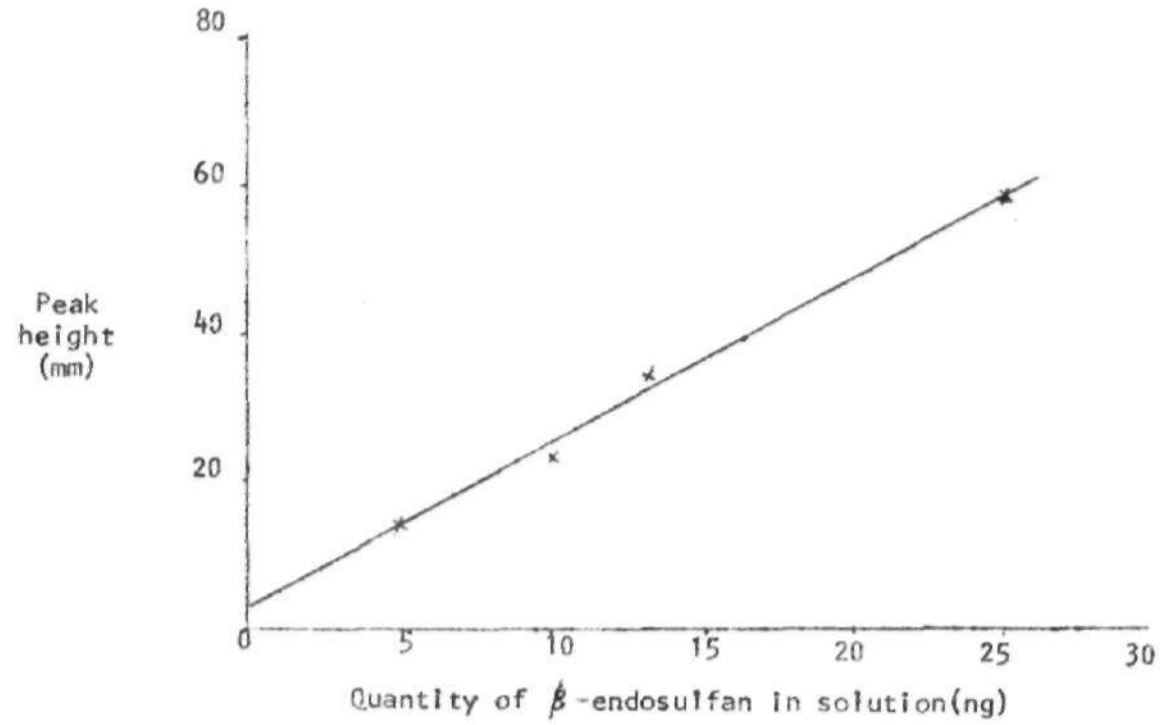


Fig. 8 Calibration Curve for β-endosulfan

Quantity of β-endosulfan ng	5	10	12.8	25	50
Peak height mm	14	22	33	58	77



3.3 Determination of Recovery Efficiencies for BHC, DDT and Endosulfan by the EPA Method

Having chosen the EPA analytical method for this work the efficiency of the method was checked in the experiment below. (See details of the EPA Method below under the analysis of the lake samples).

The reagents used for all the experiments were of the AnalaR grade and were purified appropriately. All solvents were purified by fractional distillation and the purity of the distillate tested by injecting into the gas chromatograph. All glasswares were washed with teepol, rinsed with deionized water, dried, then rinsed with dichloromethane, and dried in the oven.

1 mg each of ɣBHC, o,p' DDT, p, p' DDT, ∝-endosulfan and β-endosulfan was added to 1.5L of distilled-and-deionized water in a 2L volumetric flask. The contents were thoroughly mixed. They were then taken through all the extraction and clean-up procedures, and the amount of the different pesticides recovered was determined. From these, their different percentage recoveries were computed.

The experiment also gave information on the partitioning of the pesticides between the two elution fractions collected.

Compound	Recoveries in Percent		
	F I	F II	Total
ɣBHC	78	8	86
o,p'DDT	92	-	92
p,p'DDT	92	-	92
α- endosulfan	56	14	70
β- endosulfan*	-	300	300

Table 8: Percentage revoveries of added pesticides in water.

*For the calibration curve, β-endosulfan solution in n-hexane was used, but in the above experiment β-endosulfan was determined from fraction II (F II) which was eluted with 60:40 v/v benzene - n-hexane. The effect of these solvents on the peak height of the pesticide was not known. An attempt was made to prepare a new calibration curve for β-endosulfan using the benzene - hexane solvent but it was found that the pesticide had deteriorated from its original state, so the experiment was abandoned.

The percentage recoveries in Table 8 above compare favourably with those given in the EPA Manual[40] from which the method was adapted. Most of the losses probably resulted from incomplete extraction of the pesticides from the water in the liquid - liquid extraction method used.

The recovery of o,p' DDT in FI only and β-endosulfan in FII was a great advantage because in a mixture of both, their peaks coincide. Their elution in different fractions makes it possible to determine each without interference from the other.

3.4 Analysis of Distilled - and - Deionized Water For Pesticide Residues

This experiment was carried out in order to test the claim of the ubiquity of organochlorine residues and to know if it would be enough to rinse glassware and other apparatus for residue work with distilled-and-deionized water.

1.5L of fresh distilled-and-deionized water was used. The water was taken through the same extraction and cleanup procedures as the spiked water sample above. The "apparent" residue content of the water was then determined.

Of all the five pesticide residues analyzed for - ɣBHC, o,p' DDT, p,p' DDT, ∝-endosulfan, and β-endosulfan - only a peak corresponding to ɣBHC was identified. The amount of this apparent ɣBHC was calculated to be 0.03 ppm. This could be compared with the results of Hanock et al (1955);[21] Mcneil et al (1977)[18], Yamato et al (1978).[19]

These results show that a meticulous cleanliness should be maintained in all residue analytical work and that all glassware and other apparatus must be rinsed with an appropriate solvent in order to prevent contamination.

3.5 Analysis of A Pilot Sample from the IAR Lake

A pilot sample of 2.5 L was collected from the grassy portion of the lake.(See Fig. 4). The sample was stored in the refrigerator at $4^{o}C$ for 49 days. 1.5 L volume of the water was then taken for residue determination.

The analysis showed the presence of no detectable quantity of any of the five residues of interest. The non-detection of residues in the water after the long storage could be due to their deterioration with time. It has been demonstrated that residues in water still undergo

deterioration even under the preservation conditions.[40] After this experiment, a decision was then made that the lake samples should all be extracted the same day they were collected.

CHAPTER 4

ANALYSIS OF THE LAKE SAMPLE

EXPERIMENTALS

4.1 Solvents and Reagents

1. Methylene chloride, hexane, and benzene, all analaR grades
2. Silica gel 0.05 mm mesh. Activated for 48 hrs at 75°C before use. Deactivated material was prepared by adding 1.0ml of water to 5gm silica gel in a conical flask with a ground glass stopper and mixed for 2hrs using electric shaker. Each batch of deactivated silica gel was discarded after 5 days.
3. Sodium sulphate, granular, anhydrous, purified by Soxhlet extraction with dishloromethane.
4. Glass wool, pre-extracted with dichloromethane
5. "Keeper" solution, 1% paraffin oil in hexane.
6. Eluting solvents:

   Fraction I (FI) - hexane

   Fraction II (FII) - benzene-hexane (60:40 v/v)
7. Distilled-and-deionized water
8. Pesticide reference standards, supplied by Plant Pathology Department, IAR, ABU, Zaria.

All the reagents were AnalaR grade; and all solvents were purified by fractional distillation and their purities tested in the chromatograph before use.

4.2 Experimentals

Sample Extraction and Cleanup

The extraction and cleanup method used was a modification of the EPA method (1977).

S1

The water sample which was collected the same day was taken from the refrigerator and shaken to disperse settled particles. 1300 ml of the water was trasferred into a 2L separatory funnel. 10 gm of anhydrous sodium sulphate and 75 ml dichloromethane were added and the mixture was shaken vigorously for 2 mins. and allowed to settle. Phase separation occurred after 20 minutes, with the dichloromethane as the lower phase. There was an emulsion at the dichloromethane-water interface, and because of this a sodium sulphate column could not be used for drying the extract as this would clog the column. Instead a glass wool column was used to trap the emulsion. The extract (the organic phase) was run directly into the drying column from the separatory funnel and the dried extract was collected in a 100 ml conical quickfit flask. 75 ml of the

solvent was used to extract the sample bottle. The solvent was transferred to the aqeous phase in the funnel and the extraction process was repeated. While the flask was left to stand for phase separation, the volume of the dried extract was reduced with a rotary evaporator. The concentrator tube was inclined at about 20° to the vertical and the flask containing the extract was half immersed in a water bath previously set at 35°C. The rotator was adjusted to a slow spin and the pressure in the evaporator was reduced to 300 in.Hg. Under these conditions, there was a gentle boiling of the dichloromethane without bumping. When the volume of the extract was reduced enough so that the flask could take the second extract the evaporation was stopped. The dried second extract was collected in the same flask as the first one. The glass wool (with the trapped emulsion) was washed with 20 ml dichloromethane and the washing also collected. The extract was again concentrated in the rotatary evaporator under the same conditions as before. The evaporation was stopped when the volume of the orange-coloured extract was reduced to about 4 ml. The concentrated extract was quantitatively transferred to a graduated conical centrifuge tube and the volume was further

reduced by passing a gentle current of nitrogen gas delivered through a glass tube clamped over it. The final volume of the extract was 0.5 ml.

Silica Gel Fractionation

The silica gel fractionation column was set up and 10 ml of hexane was run through the column as a prewash and the eluate discarded. During the prewash the flow was set at 24 drops per minute. When the last of the prewash hexane reached the top of the sodium sulphate in the column, a 15 ml conical centrifuge tube containing 10 drops of the paraffin hexane keeper solution was placed under the column. Using a disposable pipet, the 0.5 ml extract was tranferred to the column being careful not to lose any drop. When this had sunk into the bed, the wall of the centrifuge tube was rinsed with 1 ml of hexane, and the washing was transferred to the column with the same disposable pipet. The washing was repeated twice more and finally 6.5 ml hexane was added to the column. The resulting 10 ml effluent was labelled SI F1 to indicate that it was the first fraction of the first sample. Subsequent fractions were appropriately labelled, according to the same scheme.

After collecting the first fraction, another 15 ml conical centrifuge tube was positioned for the collection of the second fraction (SI FII). Elution was done with the benzene - hexane (60:40 v/v) eluting solvent.

The two fractions were separately concentrated by passing a gentle current of nitrogen gas over them. Both fractions were reduced to final volumes of 1.0 ml. This final volume was dictated by the peak height obtained by injecting 1 /*l samples drawn at intervals from the extract during the concentration into the gas chromatograph.

Samples, II and III (SII, SIII)

The same procedures used for extraction, cleanup and concentration of SI were used for SII and SIII.

Table 9: Volumes of the lake water samples, and the concentrated extracts.

Sample	Volume (ml)
SI	1800
SI FI	1.0
SI FII	1.0
SII	2020
SII FI	1.0
SII FII	1.0
SIII	1900
SIII FI	3.0
SIII FII	1.8

Determination

The identification of the residues present was done by comparing the retention times and peak shapes with those of the standards. For quantitation, the extracts were diluted to get peak heights that could be read from the calibration curve. When this had been achieved, the average height of three peaks got by injecting the same quantity of extract was used to calculate the amount of residues present in the extract.

4.3 Results

Table 10: The distribution of residues in the different fractions.

Sample	Residues Present				
	∑ BHC	o,p' DDT	p,p' DDT	∝ Endo-sulfan	β-Endo-sulfan
SI FI	+	-	-	+	-
SI FII	+	-	-	+	-
SII FI	+	-	-	+	-
SII FII	+	-	-	*	-
SIII FI	+	-	-	*	-
SIII FII	+	-	-	*	-

+ ≡ residue present,
- ≡ absence, and
* ≡ presence of interference.

Quantitation

Details of the method used to calculate the γ BHC residue in Sample I (SI) is given below and the same method was used to calculate the residue of all the samples, Table 11.

SI FI

Final volume of extract = 1.0

Volume of extract injected = 1 µl

BHC peak height = 39mm

From γ BHC calibration curve, 39mm ≡ 2ng γ BHC

∴ the 1.0 µl sample, injected contains 2ng γ BHC

The whole 1.0 ml SIFI extract

∴ contains:

(2 x 1000)ng 2000 ng γ BHC

SI FII

Final volume of extract = 1.0ml

Volume of extract injected = 2 µl

BHC Peak height ... ... = 15mm.

From the calibration curve 15mm ≡ 0.9ng γ BHC

∴ the 2 l sample injected contains ... 0.9ng γ BHC

0.45ng γ BHC/µl

The whole 1.0ml SI FII

∴ contains:

$$(0.45 \times 1000)\text{ng} = \underline{450\text{ng } \gamma\text{ BHC}}$$

The total amount of γ BHC in SI = γ BHC in SI FI + γ BHC in SI FII

= (2000ng + 450 ng) γ BHC

= 2450ng γ BHC in the 1.80L water sample

$$2450\text{ng}/1.80\text{litres} = \underline{0.00136\text{ ppm}}$$

$$= 1.36\text{ ppb}$$

Table 11: The determined quantities of residues in the lake water samples

Sample	SI (1800ml)		SII (2020ml)		SIII (1900ml)	
Fraction	SI FI	SI FII	SII FI	SII FII	SIIIFI	SIII FII
γ BHC (ng)	2000	450	480	3000	1800	1170
o,p' DDT	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
p,p' DDT	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-endosulfan	1300	100	600	150*	I.D.	I.S.
β-endosulfan	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total Concentration						
γ BHC (ppb)	1.36		1.70		1.60	
α-endosulfan (ppb)	0.78		0.37		I.S.	

N.D. nondetectable
I.S. presence of interfering substances

* : In the SII FII the presence of interfering substances made the determination of α-endosulfan impossible. Theoretical calculations were therefore made based on the distribution of α-endosulfan in FI and FII as determined in a previous experiment, Table 3

The values for the residues in the three lake samples - SI, SII and SIII did not indicate the presence of any stratification in the lake water with reference to pesticide residue distribution (See Table 11). The result was therefore treated with the assumption that the residue distribution in the lake water was uniform. A simple average of the values for the three samples was therefore taken for each detected residue. This gave

1.55 ppb for βBHC, and

0.58 ppb for α-endosulfan.

4.4. Discussion and Conclusion

The results of the analysis of the lake water presented above should be considered in the light of the following:

It was only one part of the entire aquatic system that was studied, i.e. the water only. Suspended particles, bottom sediments and the biotic life were not included. The water samples were not filtered to rid them of suspended matter but the extraction method used (liquid - liquid extraction) is known not to be good for the extraction of residues adsorbed on suspended particles. It has been shown that these particles and bottom sediments contain higher levels

of residues than the water itself because of the adsorption properties of these chemicals. Also later work is needed to determine the residue levels in the animals (especially fishes) and plants associated with the lake water.

In the light of the above, the levels of BHC, DDT, and endosultan in the IAR Lake can be said to be within the safe limit when compared with specifications for potable water and with other results obtained for both polluted and unpolluted waters in different parts of the world. (See Tables 1,2,3,12,13.). It was also gathered that there has not been any incidents of massive fish deaths in the lake (another indicator of pesticide pollution). Based on the above facts, it was therefore concluded that there is no gross pollution of the IAR Lake by any of the organochlorines whose residues were analyzed for.

Table 12: Levels of residues allowed in streams for fishing (From Organochlorine Insecticides: Persistent Organic Pollutant pp. 12)

Pesticide	Limit (mg/L)
DDT	< 0.002
Endrin	< 0.004
BHC	< 0.004
Methyl parathion	< 0.100
Malathion	< 0.160

Table 13: Comparison of Drinking water Standards
(From J. Chrom. 132 p. 285 (1977)

	Maximum Permissible level (ppm).		
	Canada NH & W . (1966)	US EPA	
		1972	1975
Aldrin	0.017	0.001	0.001
Chlordan	0.002	0.003	0.003
DDT	0.042	0.05	0.05
Dieldrin	0.017	0.001	0.001
Endrin	0.001	0.005	0.002
Heptachlor	0.001	0.001	0.001
Heptachlor epoxide	0.018	0.0001	0.0001
Lindane	0.0.056	0.005	0.004

REFERENCES

1. Brooks, G.T.,1974. Chlorinated Pesticides. Vol. I. Technology And Application pp. 7, 85. CRC Press, Inc. Ohio.

2. West, T.F., and Hardy, J.E., 1961. Chemical Control of Insects. 2nd Ed. p. 92. Chapman and Hall Ltd. London.

3. Cremlyn, R.L., 1978. Pesticides: Preparation and Mode of Action pp. 1-19. John Wiley, N.Y.

4. Carson, R.L., 1962. Silent Spring. Hamish Hamilton Pub., London.

5. Gunn, D.L., and Stevens, J.G.R.,1976. Pesticides and Human Welfare. p. 118. Oxford Univ. Press, London.

6. Suffet, I.H., 1977. Fate of Organic Pollutants in the Air and Water Environments. Part III. pp. 267, 328. John Wiley and Sons, N.Y.

7. Holden, A.V., 1975. "Monitoring Persistent Organic Pollutants", Organochlorine Insecticides. pp. 11-12 Ed. Moriaty, F. Acad. Press, London.

8. Fleming, W.E., and Maines, W.W., Persitence of DDT in Soil of The Area Infected by the Japanese Beetle. J. Econ. Entomology 46 (3) pp. 445-9. (1953).

9. Lichenstein, E.P., DDT Accumulation In Mid-Western Orchard And Crop Soils. J. Econ. Entomology. 50 (5) pp. 545-7 (1957).

10. Metcalf, R.L., 1955. Organic Insecticides. p. 215. Interscience Pub. N.Y.

11. Matsumura, F., Boush, G.M., and Misato, T. (Ed.). Environmental Mutagenesis And Repair. Ann. Rev. Biochem. 42. pp. 259-78 (1978).

12. Mcewen, F.L., and Stephenson, G.R., 1979. The Use And Significance of Pesticides In The Environment. p. 410. John Wiley and Sons, N.Y.

13. Ashworth, R.D., and Henriet, J., 1970. CIPAC Handbook: Analysis of Technical And Formulated Pesticides. CIPAC Ltd., England.

14. Gunter, Z., (Ed). 1972. Analytical Methods For Pesticides And Plant Growth Regulators. VI Gas Chromatographic Analysis. Acad. Press.

15. Matsumura, F., Boush, G.M. and Misato T., (Ed.). 1972. Environmental Toxicology of Pesticides. p. 234. Acad. Press N.Y.

16. Bong, R., Collaborative study of the Recovery of BHC and Mirex in Butterfat and Fish AOAC, 60 (1) pp. 229-32. (1977).

17. Coburn, J.A., Valdamanis, I.A., and Chau, A.S.Y., Evaluation of Xad-2. For Multiresidue Extraction of Organochlorine Pesticides and PCBs from Natural Waters. AOAC 60 (1) pp. 224-8 (1977).

18. Mcneil, E.E., and Otson, R., Determination of Chlorinated Pesticides In Potable Water. J. Chrom. 132 pp. 277-86. (1979).

19. Yamato, Y., Suzuki, M., and Watanebe, T., Extraction of BHC Isomers From Water Samples Using a Macroreticular Resin. AOAC 61 (5) pp.1135-8 (1978).

20. Sunshine, I., (Ed). 1969. Handbook of Analytical Toxicology, CRC Pub., Ohio.

21. Hanock, W., and Law, E.Q. The Determination of Traces of Benzenehexachloride In Water And Sewage Effluents. Analyst 80 pp. 665-74 (1955).

22. Ahmad, N., Cleanup of biological Samples for Determining p,p´ DDT and Its Metabolites. AOAC 62 (5) pp. 1150-4 (1979).

23. Middelem, C.H. 1971. "Assay Procedures for Pesticides Residues", Pesticides In the Environment. Vol. I, Part II, p. 394. Ed. Robert, W.S. Marcel Dekker, Inc. N.Y.

24. Boyd, W.G. Jr., Gas-Liquid Chromatography of Polstar Formulations. AOAC 62 pp. 742-5 (1979).

25. Gunter, Z., and Archer, T.E., Quantitative Determination of Thiodan By Gas Chromatography. J. Agr . Food Chem. 8 pp. 190-2 (1960).

26. Koons, J.R., Simultaneous Quantitative Determination of Lindane and DDT By Gas Chromatography. J. Agr. Food Chem. 12 p. 550 (1964).

27. Gunter, Z., Archer, T.E., and Daniel, R., Residue Analysis of a Chlorinated Insecticide (Thiodan) by combination of Gas chromatography and infrared spectroscopy. J. Agr . Food Chem. 8 pp. 403-5 (1960).

28. Westlake, W.E., 1971. "Gas Chromatographic Measurement and Identification of Pesticide Residues With Electron Capture, Microcoulometric and Electrical Conductivity Detectors", Pesticide Identification At The Residue Level. pp. 73-80. American Chemical Society, Washington.

29. Ernest, J.B., Hal, H., and Keene, P.D., Gas Chromatographic Retention Times and Sensitivity Data for Insecticides And Herbicides. J. Agr. Food Chem. 12 pp. 333-6 (1964),

30. Johnson, D.P., Determination of Sevin Insecticide Residue In Fruits and Vegetables. AOAC 47 pp. 283-6 (1964).

31. Walter, R.B., and Josephine, M.F., Rapid Procedure for Carbaryl Residue: Modification of official Colorimetric Method. AOAC 48 pp. 676-9 (1965).

32. Lillian, B., and Jay, C.M., Microdetermination of Thiodan Residues. J. Agr. Food Chem. 10 pp. 479-82. (1962).

33. Jay, C.M., and Kenneth, C.W. An Improved Colorimetric Method for Determining Endosulfan Residues In Vegetables And Beef Fat. J. Agr. Food Chem. II pp. 416-8 (1963).

34. Payne, W.R. Jr., and William, S.C., Micro - IR Residue Analysis of Dieldrin, Endrin, And other Chlorinated Pesticide Residues In complex substrates. AOAC 49 pp. 989-99 (1966).

35. Blinn, R.C., IR Techniques Useful In Residue Chemistry. AOAC 48 pp. 1009-17 (1965).

36. Chen, J.T., Micro KBr Technique of IR spectrophotometry. AOAC 48 pp. 380-4 (1965).

37. Williams, B., 1978. A Sampler On Sampling Chap. II. John Wiley, N.Y.

38. Vic, B., 1974. Elements of sampling Theory. pp. 90-108. The English Univ. Press Limited.

39. Wilson, A.L., 1976. The Chemical Analysis of Water: General Principles And Techniques. p. 19. The Chemical Society, London.

40. Watts, R.L., 1977. EPA Manual of Analytical Methods for the Analysis of Pesticides in Human and Environmental Samples. Section 10, A. EPA-600/8-80-038.